

Pollen Genetic Markers for Detection of Mutagens in the Environment

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To utilize and exploit pollen for *in situ* mutagen monitoring, screening and toxicology, the range of genetic traits in pollen must be identified and analyzed. Traits that can be considered include ornamentation, shape and form, male sterility viability, intraspecific incompatibility, proteins and starch deposition. To be useful for the development of mutagen detection systems proteins should be: (1) activity stainable or immunologically identifiable in the pollen, (2) the products of one to three loci, and (3) gametophytic and nuclear in origin. Several proteins including alcohol dehydrogenase in maize, which meet those criteria will be discussed. The waxy locus in barley and maize which controls starch deposition has been characterized genetically and methods have been developed for pollen screening and mutant detection. At Washington State University a waxy pollen system is being developed in barley for *in situ* mutagen monitoring. The basis is an improved method for staining and scoring waxy pollen mutants. Specific base substitution, frameshift, and deletion mutant lines are being developed to provide information about the nature of the mutations induced by environmental mutagens. Thirty waxy mutant lines, induced by sodium azide and γ -rays have been selected and are being characterized for spontaneous and induced reversion frequencies, allelism, karyotype, amylose content, and UDP glucose glucosyltransferase (waxy gene product) activity. Twelve mutant alleles are being mapped by recombinant frequencies.

Introduction

Appropriate systems to monitor *in situ* the genetic hazards of environmental pollutants to man, animals, and plants, are very rare. Indeed, among higher eukaryotes only two systems, both plants, have been used with any success (1). This paper is concerned with improving the capability of plants for *in situ* monitoring of genetic effects of the environment.

In developing an *in situ* monitoring system it is first necessary to examine the criteria for such a system. An appropriate system should include: chromosome organization and molecular biology similar to that of the human organism, i.e., a eukaryote; well-defined and simply inherited genetic markers or traits; ability to provide reliable mutation frequency data from different stages in the life cycle at very low doses of environmental

mutagens; capability for measuring chronic exposures; a means for providing data on the nature of mutations, especially at the DNA level; short time between mutation induction and mutant screening; relatively low cost of culture and cost and time of training technicians to score genetic endpoints following mutagen treatment; small space requirement for mobile atmospheric testing laboratories and growth controlled chambers; and ability to grow under a wide range of atmospheric and soil conditions.

Plants meet many of these criteria and have a number of advantages over mammalian and prokaryotic systems for environmental mutagen monitoring (1). In addition to the advantages over mammalian systems, plants possess a pollen genetic system.

Pollen has several attributes for measuring biological damage when used as a monitor for air or soil pollution. It is haploid so that the genetic changes occurring in plants up to the time of complete development and final mitosis can be

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scored directly. This permits chronic exposure of the sporophyte to a mutagenic agent while observing the genetic effects of such exposure in the haploid germinal cell. In addition, pollen systems provide a highly sensitive means for screening and monitoring environmental mutagens since increases in genetic effects of low levels of mutagens can be recorded. Mutant traits can be quickly detected from among millions of pollen grains. This genetic resolution, unique among higher eukaryotes and paralleling that of prokaryotes, permits the scoring of intragenic events—thus providing an understanding of the nature of induced mutations in higher plants. Further advantages include low cost and ability to measure genetic damage at different stages of pollen development in terms of reduction of pollen germination, chromosome aberrations [fragments and dicentrics in pollen mitosis, micronuclei in tetrads (2) and nondisjunction (3)] and mutants of morphological and biochemical traits. Such traits or markers will now be described.

Potential Pollen Genetic Markers

To fully utilize and exploit pollen in mutagen monitoring, screening and toxicology, the range of genetic traits in pollen must be identified and analyzed. A reasonably extensive review of the literature has determined that there are currently available very few pollen genetic markers that could be developed for mutagen monitoring systems. This is in part due to the lack of genetic and molecular biological knowledge of many potential traits. Furthermore, the useful traits are limited to the gametophytic or haploid portion of the pollen grains. The following summarizes the status of some potential markers. They include outer wall ornamentation, pollen shape and form, male sterility, viability, incompatibility, protein variation, and starch deposition.

Ornamentation

Sculpture or ornamentation of the outer layer (tectum) of the outer wall (exine) which is readily observed under a light microscope might be considered an obviously useful genetic trait. It involves the distribution and dimension of spines, ridges, perforations, papillae and patterns of spinulas on ridges. However, it is generally recognized (4) that it is difficult to distinguish ornamentation patterns between species and even genera, and to use such patterns for taxonomic classification. This suggests that ornamentation does not involve simple genetic control. Furthermore, origin of the exine is sporophytic (5), and the ornamental sculpturing is

probably of no value in a mutagen monitor where mutants expressed in the haploid state are required.

Shape and Form

Distinct shapes and forms of the pollen are known but genetic analysis is lacking. Moreover, it is likely that the structures involved in shape and form are sporophytic in origin and that allometry is not a useful trait for measuring effects of mutagens. However, pine (*Pinus*) pollen may have some potential for the screening of environmental mutagens. This pollen normally has two attached bladders; however, pollen with three and four bladders have been observed (R. Mack, personal communication). If simply inherited and gametophytic in origin, this trait may prove useful.

Male Sterility

Genetic male sterility will lead to increased number of aborted pollen. Genes for male sterility have been identified in many plants. For instance, in barley 16 loci are known (6, 7). In such plants, forward mutations to male sterility could be identified. However, since cytoplasmic male sterility also exists and leads to aborted pollen, scoring for forward mutations could be complicated. On the other hand, reverse mutation in a homozygous genetic male sterile line could lead to normal pollen (detected by fluorescein diacetate) and seed set, both easily scored.

Viability

Viability is probably controlled by a number of genes. This could be a useful multilocus system for mutagen monitoring. Stains for revealing viable or nonviable pollen are available to quickly record mutations at these loci. Mulcahy (8) has suggested an approach for recording such mutations.

Intraspecific Incompatibility

Mutations for genes controlling incompatibility, such as the S locus in *Oenothera organensis* can be readily detected and represent a potentially powerful monitoring system. Since this subject is covered elsewhere in this symposium (9) it is not further described here.

Protein Markers

Most of the apparent useful heritable traits identified in the pollen of various plant species are

proteins. However, pollen proteins have been identified and analyzed for reasons other than utilization in a mutagen monitoring and screening system, such as to determine allergen effects, incompatibility relationships ("recognition substances"), various aspects of beekeeping, and to identify varieties and subvarieties by isozyme patterns (10).

Numerous proteins, mostly enzymes, have been identified in pollen. Most were described by Brewbaker (11) and all are listed in Table 1. Most

are incorporated in the pollen wall and a major problem in analysis is to determine if a given protein is in the sporophytic or gametophytic portion of the pollen (5). Proteins incorporated within the inner wall or intine are gametophytic in origin, while those associated with the outer wall or exine are sporophytic in origin (5). The cell wall proteins, consisting of antigens as well as enzymes, are freely diffusible with the sporophytic proteins being released first (5).

The time of synthesis and incorporation of pro-

Table 1. Enzymes reported to be active in pollen grains.

Enzyme group	Enzyme trivial name	Reference
Dehydrogenases	Alcohol dehydrogenase	(12)
	Glucose 6-phosphate dehydrogenase	(11)
	Glutamate dehydrogenase	(11)
	Isocitrate dehydrogenase	(11)
	Lactate dehydrogenase	(11)
	Lipoamine dehydrogenase	(11)
	Malate dehydrogenase	(11)
	6-Phosphogluconate dehydrogenase	(11)
	Succinate dehydrogenase	(11)
	Triosephosphate dehydrogenase	(11)
	UDPG dehydrogenase	(11)
Oxidases	Amino acid oxidase	(11)
	Catalase	(11)
	Cytochrome oxidase	(11)
	Peroxidase	(11)
Transferases	ADPG glucosyltransferase	(13)
	ADPG pyrophosphorylase	(11)
	Alanine aminotransferase	(11)
	Aspartate aminotransferase	(11)
	Aspartate carbonyltransferase	(11)
	Hexokinase	(11)
	Nucleoside diphosphate kinase	(11)
	Phosphoglucomutase	(11)
	Phosphorylase	(11)
	Ribonuclease	(11)
	Sucrose synthetase	(13)
	Trehalose 6-phosphate synthetase	(14)
	UDPG pyrophosphorylase	(11)
	UDPG glucosyltransferase	(13)
	UDPG: quercetin glucosyltransferase	(15)
Hydrolases	Acid phosphatase	(11)
	Alkaline phosphatase	(11)
	Aminoacylase	(11)
	Amylase	(11)
	Cellulase (β -1,4-glucanase)	(11)
	Cutinase	(16)
	Esterases	(11)
	β -Fructofuranosidase (invertase)	(11)
	α -Glucosidase	(11)
	Leucine aminopeptidase	(11)
	Pectinase	(11)
	Protease (trypsin and chymotrypsin)	(11)
	Trehalase	(11)
	Trehalose 6-phosphate phosphatase	(14)
Lyases	Citrate synthetase	(11)
Isomerases	Ketose 1-phosphate aldolase	(11)
	Glucosephosphate isomerase	(13)
Ligases	Carboxylases	(11)

teins in the pollen is important in relation to mutation induction and detection. It appears that proteins are synthesized and incorporated soon after the release of microspores from the tetrads (5, 17).

Most proteins have not been characterized genetically nor localized within the pollen grains. Therefore, much more work is necessary before their potential use in mutagen monitoring systems can be evaluated. Nevertheless, several protein pollen markers as described below have some promise. These markers are: activity stainable or immunologically identifiable in intact pollen grains, the product of one to three genetic loci, and gametophytic and nuclear in origin. Such characteristics facilitate detection and screening of genetic variants.

Acid phosphatase is perhaps the most widely studied of the pollen protein markers (18-25). It has been localized in the intine (18, 23-25) and is thought to be controlled by three unlinked loci, AP_1 , AP_2 and AP_3 in maize (20-22). Acid phosphatase activity is stainable by incubating pollen 1-5 min with α -naphthyl acid phosphate coupled with hexazonium pararosanilin to give a reddish-brown color (26). The substrates naphthol S-B1 or AS-TR phosphates can also be used; however, the reaction is not as rapid (25). In addition, Fast Garnet GBC can be used as a coupler to yield a purple-black color and has been shown to specifically inhibit AP_3 (19).

Three AP_1 isozymes occur in maize pollen (20). The enzyme appears to be a dimer and is synthesized after meiosis since pollen from heterozygous plants lack hybrid enzyme. Segregation ratios of AP_1 isozyme activity levels showed that the isozymes are controlled by a single gene.

Leucine aminopeptidase is widely distributed in angiosperm pollen (10). In maize endosperm there are apparently four genes involved (27). However, in pollen it appears that only one to three are expressed for a given species (10, 11). Genetic polymorphisms occur in maize pollen for the LP_2 locus (11). Electrophoretically separated proteins are activity stained by using L-leucyl- β -naphthylamide HCl as a substrate and Black K salt, a diazonium dye, as a coupler, to yield a deep purple color. Such a procedure might be useful for pollen staining, but as yet the enzyme has not been localized.

α -Amylase (Amy) is apparently under the control of a single gene in maize (28) and barley (29, 30). In barley there are at least three alleles at the Amy locus which give rise to the three isozymes, α -type 1, α -type 2, and α -type 3. Jones and Chen (31) used immunofluorescent antibodies to localize α -amylase in barley aleurone cells. It should be possible to use a similar method for detecting α -amylase in pollen. Amylase has been shown to be readily diffusible

from birch pollen after 1 min, indicating it may be sporophytic in origin (32). However, Knox and Heslop-Harrison (25) report that the activity was localized in the intine. Further work to localize the enzyme accurately is needed.

The alcohol dehydrogenase (*Adh*) locus in maize (33-35) is controlled by two unlinked genes, *Adh1* and *Adh2*. The functional enzyme can be found electrophoretically as either a homodimer or a heterodimer of the gene products of the *Adh1* and *Adh2* loci. The maize alcohol dehydrogenase has been purified and partially characterized (35) and numerous mutant enzymes have been electrophoretically characterized by Schwartz (33, 36). Only *Adh1* is expressed in the pollen grain, and most of the *Adh1* polypeptides are synthesized after anaphase II of meiosis (37). The use of maize *Adh1* as a monitor of environmental mutagens has been discussed by Freeling (35) and is reviewed further in this publication (38).

There are several pollen wall antigens which may be useful for mutagen monitoring. Of these, antigen E is perhaps the best characterized. Immunofluorescent antibodies (39) revealed antigen E of ragweed (*Ambrosia*) to be localized to the intine. Antigen E was localized to the intine and exine of the pollen of *Cosmos* (40). With fresh pollen, much of the antigenic material was discharged into the medium; therefore, it may be necessary to use frozen pollen for effective immunofluorescent detection and screening.

Starch Deposition (Waxy)

The waxy trait has been observed in over 15 genera of angiosperms and is characterized by failure of amylose deposition in the endosperm and pollen grain starch (41). Starch granules in the cereal pollen grain begin to appear just after the second pollen mitosis (42) and are gametophytically controlled (41). Waxy and nonwaxy pollen stain differentially with iodine, blue for pollen with normal starch and amylose content and reddish-brown for pollen with amylopectin and no amylose. All of the early genetic research on waxy pollen has been reviewed by Eriksson (41). Genetic studies in maize, barley, rice, and sorghum concluded that the waxy character is inherited as a monofactorial recessive.

The waxy locus offers several distinct advantages for a mutagen monitoring system. Because mutants are identified by iodine staining, the procedure is relatively simple and not as time consuming as procedures requiring cumbersome electrophoretic detection methods. In addition, the pollen screening procedure is much less costly than those

requiring complex reagents or immunochemicals for mutant identification.

Some knowledge of the biochemistry of the waxy gene product is available. Waxy mutants in maize completely lack starch granule-bound uridine diphosphoglucose (UDPG) glucosyltransferase activity (43) and enzyme activity is linearly proportional to the number of starchy (*Wx*) alleles present in the endosperm (44). These results support the suggestion that the waxy locus is the structural gene for the UDPG glucosyltransferase.

The pollen waxy locus of maize has been used to detect the genetic effects of herbicides and is being developed into a mutagen monitoring system (45, 46). This system is described in detail in these proceedings (47).

Development of a Waxy Pollen System in Barley

The development of a waxy pollen system in barley for mutagen monitoring (both frequencies and nature of events) has been underway at Washington State University for several years. Included in the overall objective is to develop a genetic system that is highly sensitive to low levels of mutagens and that can measure the frequencies and kinds of changes at the DNA level. The attributes of the pollen system have been described above.

Barley was selected because it: (1) is genetically well characterized, (2) is a self-pollinated diploid, (3) has seven pairs of relatively large chromosomes, (4) is easy to culture and grows under a wide range of climate and soil conditions, (5) is small in stature and many plants can be confined to laboratories and controlled growth chambers, (6) exhibits some meiotic synchrony, and (7) is the foremost of all plants in terms of studies on experimental mutagenesis (48-50). The synchrony of meiosis permits treatment of microspores and scoring

for waxy mutants and chromosome aberration in pollen at precise times. It is also adaptable for *in situ* monitoring of genetic activity in the environment in and around laboratories, factories, nuclear reactors and waste chemical dump sites, and of chemicals involved in agricultural practices. Mutations at the waxy locus in barley were used to detect the mutagenic effect of ethylene oxide in air around Stockholm (51).

A key to the development of this system is a rapid mass-screening technique for analyzing waxy mutants of pollen (52). It permits the analysis by one recorder of one million pollen grains from 15 different spikes in an 8-hr day and has led to reliable data for forward mutations and mutant allele revertants and recombinants.

Mutant lines induced by base substitution (sodium azide, ethyl methanesulfonate), frameshift (acridine, proflavin), and deletion (ethyl methanesulfonate, γ -rays) mutagens are being selected and developed to provide information about the nature of the mutations induced by environmental mutagens and to provide a broad base for detecting different mutagens.

To date, about 30 mutant lines induced primarily by sodium azide and gamma rays have been selected. These are being characterized for spontaneous reversion rates, amylose content, enzyme activity, and karyotype. They will also be characterized as to reversion frequencies induced by the different mutagen types.

Sodium azide is probably the best candidate in barley for a base substitution mutagen. It does not induce chromosome breaks in barley nor sister chromatid exchanges in mammalian cells (53). However, it induces very high frequencies of chlorophyll-deficient mutations in barley and has been shown to be a base substitution mutagen in *Salmonella* (54). It is a major environmental mutagen as it is used in medicine, agriculture, auto air bags, and airplane escape chutes.

Table 2. Spontaneous reversion frequencies of putative base-substitution mutants.

Mutant allele	Origin	Year	Number of pollen screened	Reversion frequency $\times 10^{-5}$
Az 24	Azide-induced	1980	337,720	9.2
33	"	1979	508,820	17.1
	"	1980	1,015,016	20.7
35	"	1979	996,160	41.2
	"	1980	727,360	12.9
44	"	1979	357,620	16.7
	"	1980	1,179,360	10.9
45	"	1979	428,800	10.0
	"	1980	529,280	8.7
48	"	1979	351,800	8.2
	"	1980	471,840	6.8

Table 3. Spontaneous reversion frequencies of putative deletion mutants.

Mutant allele	Origin	Year	Number of pollen screened	Reversion frequency $\times 10^{-5}$
$\gamma 3$	γ -Induced	1979	494,900	17.2
	"	1980	1,704,440	17.2
4	"	1980	336,400	22.6
5	"	1980	1,096,128	6.2
6	"	1980	1,227,760	23.6
7	"	1980	1,094,640	7.3
8	"	1980	1,149,480	31.6
9	"	1980	943,880	30.6

Spontaneous frequencies of some waxy mutant lines induced by sodium azide are shown in Table 2. For the most part, the reversion frequencies are high but stable from year to year. Among these mutant lines, Az 24 and Az 44 appear to have the most stable baseline reversion frequency since little difference in reversion frequency is observed between plants within these mutant lines.

Spontaneous reversion frequencies from some lines induced by γ -irradiation are shown in Table 3. These frequencies range from 6.2×10^{-5} to 31.6×10^{-5} . The mutant line gamma 5 appears to have the most stable baseline reversion frequency and is the most promising putative deletion line thus far available.

Chemical and karyotype characterization of the waxy mutant lines has made considerable progress. Twenty-one azide-induced and seven γ -ray-induced waxy mutants were analyzed for amylose content and none contained this compound. In addition, six γ -induced and two azide-induced waxy mutants were analyzed for UDPglucose glucosyltransferase activity. One azide-induced mutant showed substantial activity (36% of normal); however, this might possibly be due to the presence of contaminating nonendosperm tissue in the starch extract.

Examinations of chromosome morphology during somatic metaphase and meiosis have revealed no major change in several azide-induced mutants, but some possible chromosome changes in two γ -ray induced mutants.

Six mutant waxy alleles were preliminarily mapped in 1979 from allelic recombination frequencies (55). Recombination frequencies from additional crosses among these plus six other alleles are now being obtained for more comprehensive mapping of the locus.

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REFERENCES

1. Nilan, R. A. Potential of plant genetic systems for monitoring and screening mutagens. *Environ. Health Perspect.* 27: 181 (1978).
2. Ma, T.-H. Micronucleus system in *Tradescantia* pollen mother cells as an index of chromosome aberration production. *Environ. Health Perspect.* 37: 85 (1980).
3. Weber, D. F. Maize pollen grains as a screen for non-disjunction. *Environ. Health Perspect.* 37: 79 (1980).
4. Köhler, E., and Lange, E. A contribution to distinguishing cereal from wild grass pollen grains by LM and SEM. *Grana* 18: 133 (1979).
5. Heslop-Harrison, J. The physiology of the pollen grain surface. *Proc. Roy. Soc. (London)* B190: 275 (1975).
6. Hockett, E. A., and Eslick, R. F. Genetic male sterility in barley. I. Nonallelic genes. *Crop Sci.* 8: 218 (1968).
7. Hockett, E. A., and Eslick, R. F. Genetic male-sterile genes useful in hybrid barley production. In: *Barley Genetics II*. R. A. Nilan, Ed., Washington State University Press, Pullman, Wash., 1971.
8. Mulcahy, D. L. Pollen in tetrads as indicators of environmental mutagenesis. *Environ. Health Perspect.* 37: 91 (1980).
9. Ramulu, K. S., Schibilla, H., and Dijkhuis, P. The self-incompatibility system of *Oenothera organensis* for the detection of genetic effects of low radiation doses. *Environ. Health Perspect.* 37: 43 (1980).
10. Mäkinen, Y., and MacDonald, T. Isoenzyme polymorphism in flowering plants. II. Pollen enzymes and isoenzymes. *Physiol. Plant.* 21: 477 (1968).
11. Brewbaker, J. L. Pollen enzymes and isoenzymes. In: *Pollen Development and Physiology*. J. Heslop-Harrison, Ed., Butterworths, London, 1971.
12. Freeling, M. Intragenic recombination in maize: Pollen analysis methods and the effect of parental *Adh1*⁺ isoalleles. *Genetics* 83: 701 (1976).
13. Bryce, W. H., and Nelson, O. E. Starch-synthesizing enzymes in the endosperm and pollen of maize. *Plant Physiol.* 63: 312 (1979).
14. Gussin, A. E. S., and McCormack, J. H. Trehalase and the enzymes of trehalose biosynthesis in *Lilium longiflorum* pollen. *Phytochemistry* 9: 1915 (1970).
15. Larson, R. L. Glucosylation of quercetin by a maize pollen enzyme. *Phytochemistry* 10: 3073 (1971).
16. Maiti, I. B., Kolattukudy, P. E., and Shaykh, M. Purification and characterization of a novel cutinase from nasturtium (*Tropaeolum majus*) pollen. *Arch. Biochem. Biophys.* 196: 412 (1979).
17. Mascarenhas, J. P. The biochemistry of angiosperm pollen development. *Bot. Rev.* 41: 259 (1975).

18. Knox, R. B. Pollen-wall proteins: Localization, enzymic and antigenic activity during development in *Gladiolus* (Iridaceae). *J. Cell Sci.* 9: 209 (1971).
19. Efron, Y. Specific inhibition of acid phosphatase-3 in pollen of maize by the diazonium salt Fast Garnet GBC. *J. Histochem. Cytochem.* 17: 734 (1969).
20. Efron, Y. Tissue specific variation in the isozyme pattern of the AP₁ acid phosphatase in maize. *Genetics* 65: 575 (1970).
21. Efron, Y. Differences between maize inbreds in the activity level of the AP₁-controlled acid phosphatase. *Biochem. Genet.* 5: 33 (1971).
22. Efron, Y. Inheritance studies with inbred lines of maize having different activity levels of the AP₁ controlled acid phosphatase isozymes. *Theoret. Appl. Genet.* 43: 323 (1973).
23. Heslop-Harrison, J., and Heslop-Harrison, Y. Pollen-wall proteins: 'Gametophytic' and 'sporophytic' fractions in the pollen walls of the Malvaceae. *Ann. Bot.* 37: 403 (1973).
24. Vithanage, H. I. M. V., and Knox, R. B. Pollen development and quantitative cytochemistry of exine and intine enzymes in sunflower, *Helianthus annuus* L. *Ann. Bot.* 44: 95 (1979).
25. Knox, R. B., and Heslop-Harrison, J. Pollen-wall proteins: Localization and enzymic activity. *J. Cell Sci.* 6: 1 (1970).
26. Barka, T., and Anderson, P. J. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* 10: 741 (1962).
27. Beckman, L., Scandalios, J. G., and Brewbaker, J. L. Genetics of leucine aminopeptidase isozymes in maize. *Genetics* 50: 899 (1964).
28. Nelson, O. E., Jr., and Burr, B. Biochemical genetics of higher plants. *Ann. Rev. Plant Physiol.* 24: 493 (1973).
29. Frydenberg, O., and Nielsen, G. Amylase isozymes in germinating barley seeds. *Hereditas* 54: 123 (1966).
30. Frydenberg, O., Nielsen, G., and Sandfaer, J. The inheritance and distribution of α -amylase types and DDT responses in barley. *Z. Pflanzenzüchtg.* 61: 201 (1969).
31. Jones, R. L., and Chen, R. F. Immunohistochemical localization of α -amylase in barley aleurone cells. *J. Cell Sci.* 20: 183 (1976).
32. Laine, S. Diffusion of proteins by intact birch pollen grains: enzymic and antigenic activity. *Grana* 16: 187 (1977).
33. Schwartz, D. Dimerization mutants of alcohol dehydrogenase of maize. *Proc. Nat. Acad. Sci. (U.S.)* 68: 145 (1971).
34. Freeling, M. Allelic variation at the level of intragenic recombination. *Genetics* 89: 211 (1978).
35. Freeling, M. Maize Adh1 as a monitor of environmental mutagens. *Environ. Health Perspect.* 27: 91 (1978).
36. Schwartz, D. The molecular basis for allelic complementation of alcohol dehydrogenase mutants of maize. *Genetics* 79: 207 (1975).
37. Schwartz, D. Genetic control of alcohol dehydrogenase—A competition model for regulation of gene action. *Genetics* 67: 411 (1971).
38. Schwartz, D. *Adh* locus in maize for detection of mutagens in the environment. *Environ. Health Perspect.* 37:000 (1980).
39. Knox, R. B., and Heslop-Harrison, J. Pollen-wall proteins: localization of antigenic and allergenic proteins in the pollen-grain walls of *Ambrosia* spp. (ragweeds). *Cytobios* 4: 49 (1971).
40. Howlett, B. J., Knox, R. B., and Heslop-Harrison, J. Pollen-wall proteins: Release of the allergen Antigen E from intine and exine sites in pollen grains of ragweed and *Cosmos*. *J. Cell Sci.* 13: 603 (1973).
41. Eriksson, G. The waxy character. *Hereditas* 63: 180 (1969).
42. Chapham, D. H. Haploid induction in cereals. In: *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture*. J. Reinert and Y. P. S. Bajaj, Eds., Springer-Verlag, Berlin, 1977.
43. Nelson, O. E., and Rines, H. W. The enzymatic deficiency in the waxy mutant of maize. *Biochem. Biophys. Res. Commun.* 9: 297 (1962).
44. Tsai, C. Y. The function of the waxy locus in starch biosynthesis in maize endosperm. *Biochem. Genet.* 11: 83 (1974).
45. Plewa, M. Activation of chemicals into mutagens by green plants: A preliminary discussion. *Environ. Health Perspect.* 27: 45 (1978).
46. Plewa, M. J., and Gentile, J. M. Mutagenicity of atrazine: A maize-microbe bioassay. *Mutat. Res.* 38: 287 (1976).
47. Plewa, M. J., and Wagner, E. D. Germinal cell mutagenesis in specially designed maize genotypes. *Environ. Health Perspect.* 37: 61 (1980).
48. Nilan, R. A. The Cytology and Genetics of Barley, 1951-1962. Washington State University Press, Pullman, Wash., 1964.
49. Nilan, R. A. Barley (*Hordeum vulgare*). In: *Handbook of Genetics*, Vol. 2: Plants, Plant Viruses, and Protists. R. C. King, Ed. Plenum Press, New York, 1974, Chap. 4.
50. Nilan, R. A., and Vig, B. K. Plant test systems for detection of chemical mutagens. In: *Chemical Mutagens: Principles and Methods for Their Detection*, Vol. 4, A. Hollaender, Ed., Plenum Press, New York, 1976, Chap. 39.
51. Sulovska, K., Lindgren, D., Eriksson, G., and Ehrenberg, L. The mutagenic effect of low concentrations of ethylene oxide in air. *Hereditas* 62: 264 (1969).
52. Rosichan, J., Arenaz, P., Blake, N., Hodgdon, A., Kleinhofs, A., and Nilan, R. A. An improved method for the detection of mutants at the waxy locus in *Hordeum vulgare*. *Environ. Mutagenesis*, in press.
53. Arenaz, P., and Nilan, R. A. Effect of sodium azide on sister-chromatid exchanges in human lymphocytes and Chinese hamster cells. *Mutat. Res.*, in press.
54. Kleinhofs, A., Owais, W. M., and Nilan, R. A. Azide. *Mutat. Res.* 55: 165 (1978).
55. Rosichan, J. L. Genetic fine structure analysis of the waxy locus in barley (*Hordeum vulgare*). M. S. Thesis, Washington State University, Pullman, Wash., 1979.